Conceptual Model for Assessing Criteria Air Pollutants in a Multipollutant Context:

A modified adverse outcome pathway approach

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Alternative Approaches for Acute Inhalation Toxicity to Address Global Regulatory and Non-regulatory Data Requirements

PETA International Science Consortium (PISC), Ltd.

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

Webinar Series

July 12, 2016

Thank you....
Today I'll be talking about....

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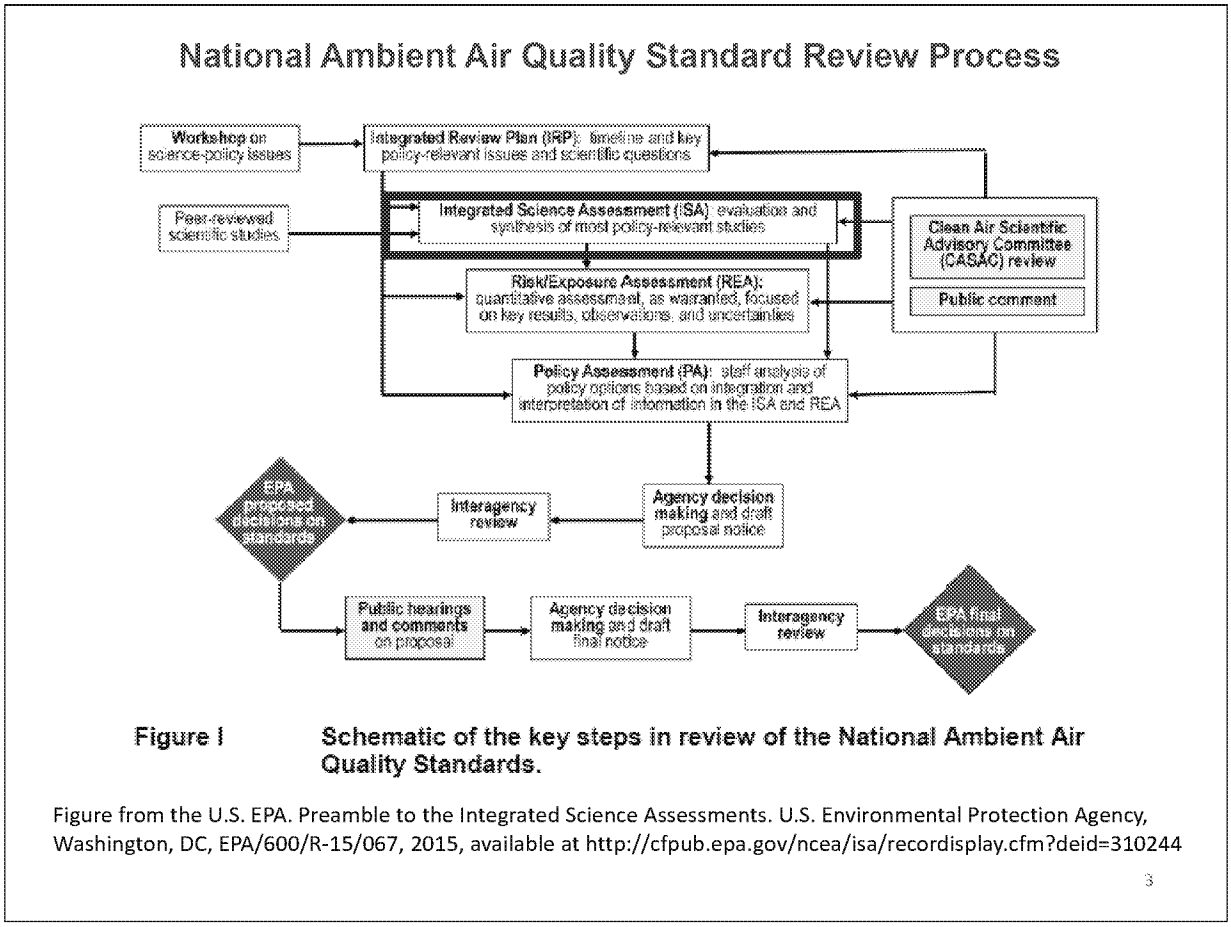

Figure I **Schematic of the key steps in review of the National Ambient Air Quality Standards.**

Figure from the U.S. EPA. Preamble to the Integrated Science Assessments. U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-15/067, 2015, available at http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=310244

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Trained in inhalation toxicology
Dissertation work - Cadmium Oxide Toxicity in the Rat Lung

20+ years research experience in redox biology
and cell signaling using in vitro models–
mainly Type 2 alveolar epithelial cells and vascular endothelial cells

8+ years at EPA evaluating the health effects of air pollutants
Specifically work on the Integrated Science Assessments for the criteria air pollutants regulated under the Clean Air Act – ozone, particulate matter, sulfur oxides, oxides of nitrogen, carbon monoxide, and lead

Development of the ISA is an early step in the review of the
National Ambient Air Quality Standards for these criteria pollutants

NAAQS are promulgated for individual criteria pollutants not mixtures of criteria air pollutants

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2004 NAS Report: "Air Quality Management in the United States"

Recommendation:

Address multiple pollutants in the NAAQS review and standard setting process

"Although the committee does not believe that the science has evolved to a sufficient extent to permit the development of multipollutant NAAQS, it would be scientifically prudent to begin to review and develop NAAQS for related pollutants in parallel and simultaneously"

NOTE: There are Currently no Plans to Attempt the Development of Multipollutant Primary NAAQS

Review

Practical Advancement of Multipollutant Scientific and Risk Assessment Approaches for Ambient Air Pollution

Douglas O. Johns,[1] Lindsay Wichers Stanek,[1] Katherine Walker,[2] Souad Benromdhane,[3] Bryan Hubbell,[3] Mary Ross,[1] Robert B. Devlin,[4] Daniel L. Costa,[5] and Daniel S. Greenbaum[2]

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OBJECTIVES: The U.S. Environmental Protection Agency is working toward gaining a better understanding of the human health impacts of exposure to complex air pollutant mixtures and the key features that drive the toxicity of these mixtures, which can then be used for future scientific and risk assessments.

DATA SOURCES: A public workshop was held in Chapel Hill, North Carolina, 22–24 February 2011, to discuss scientific issues and data gaps related to adopting multipollutant science and risk assessment approaches, with a particular focus on the criteria air pollutants. Expert panelists in the fields of epidemiology, toxicology, and atmospheric and exposure sciences led open discussions to encourage workshop participants to think broadly about available and emerging scientific evidence related to multipollutant approaches to evaluating the health effects of air pollution.

SYNTHESIS: Although there is clearly a need for novel research and analytical approaches to better characterize the health effects of multipollutant exposures, much progress can be made by using existing scientific information and statistical methods to evaluate the effects of single pollutants in a multipollutant context. This work will have a direct impact on the development of a multipollutant science assessment and a conceptual framework for conducting multipollutant risk assessments.

CONCLUSIONS: Transitioning to a multipollutant paradigm can be aided through the adoption of a framework for multipollutant science and risk assessment that encompasses well-studied and ubiquitous air pollutants. Successfully advancing methods for conducting these assessments will require collaborative and parallel efforts between the scientific and environmental regulatory and policy communities.

KEY WORDS: air pollution, exposure, human health, multipollutant, risk assessment. *Environ Health Perspect* 120:1238–1242 (2012). http://dx.doi.org/10.1289/ehp.1204939 [Online 29 May 2012]

health effects. As additional evidence of its commitment to this new thinking, scientists within the U.S. EPA's National Center for Environmental Assessment (NCEA), which is responsible for evaluating and synthesizing the scientific information related to the effects of exposure to criteria air pollutants as a part of the National Ambient Air Quality Standards (NAAQS) review process, are currently developing plans for conducting a formal multipollutant science assessment (MSA) of the health effects of exposure to air pollutant mixtures. As an initial step in the development of this proposed human health MSA, the U.S. EPA is preparing a framework describing the purpose and scope of the MSA, along with plans for conducting multipollutant analyses using existing data and information that will provide scientific support to the development of the MSA. The MSA is intended to serve as a companion document to single-pollutant Integrated Science Assessments (ISAs) of the

*Characterization of Common Modes of Action and Toxicity Pathways**

(Lead: Barbara Buckley)

Do multiple criteria pollutants act through similar pathways to induce health effects?

- *Develop framework*
- *Fit existing information for single pollutants into framework*
- *Provide case studies illustrating converging effects/converging pathways for multiple pollutants*

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Out of these efforts, came this project which I lead. The first question is which framework to use?

MOA Definition

EPA Cancer Guidelines 2005

"The term *"**mode of action**"* is defined as a **sequence of key events and processes**, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and **resulting in cancer formation**. A ***"key event"*** is an **empirically observable precursor step** that is itself a **necessary element** of the mode of action or is a biologically based marker for such an element.

Mode of action is contrasted with ***"mechanism of action"***, which implies a **more detailed understanding and description of events**, often at the molecular level, than is meant by mode of action.

The toxicokinetic processes that lead to formation or distribution of the active agent to the target tissue are considered in estimating dose but are not part of the mode of action as the term is used here.

There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immune suppression."

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Mode of Action also goes back several decades and is still in use today

Exactly what is MOA? Many definitions – sequence of key events leading to cancer formation or a toxic effect. Distinction is usually made between MOA and mechanism where mechanism implies a more detailed understanding of events at the molecular level.

Most of the outcomes of interest in the NAAQS process are non-cancer outcomes such as respiratory and cardiovascular morbidity

MOA: Cancer vs non-cancer outcomes

Bogdanffy et al., ***Harmonization of cancer and noncancer risk assessment: proceedings of a consensus-building workshop.*** Tox Sci 61:18-31, 2001

"Mode of action is a **series of key events** supported by a body of scientific knowledge that provide a biologically plausible explanation of causality for a **given toxic effect** within a context of dose and duration of exposure and susceptibility of target tissues."

"In contrast, **"mechanism"** of action refers to a **complete understanding** and demonstration of all biological steps leading to toxicity."

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So, this definition is generally more appropriate for our topic today

MOA: Experimental animals vs humans

Seed et al., ***Overview: Using mode of action and life stage information to evaluate the human relevance of animal toxicity data.*** Crit Rev Toxicol 35: 663-672, 2005

"Mode of action is defined as the sequence of key cellular and biochemical events that result in a toxic effect, while mechanism implies a more detailed understanding of the molecular basis of the toxic effect. **Complete mechanism of action information is rarely available and is not required for human health risk assessment."**

Recommends **human relevance framework** to determine whether MOA determined in animal studies is plausible in humans

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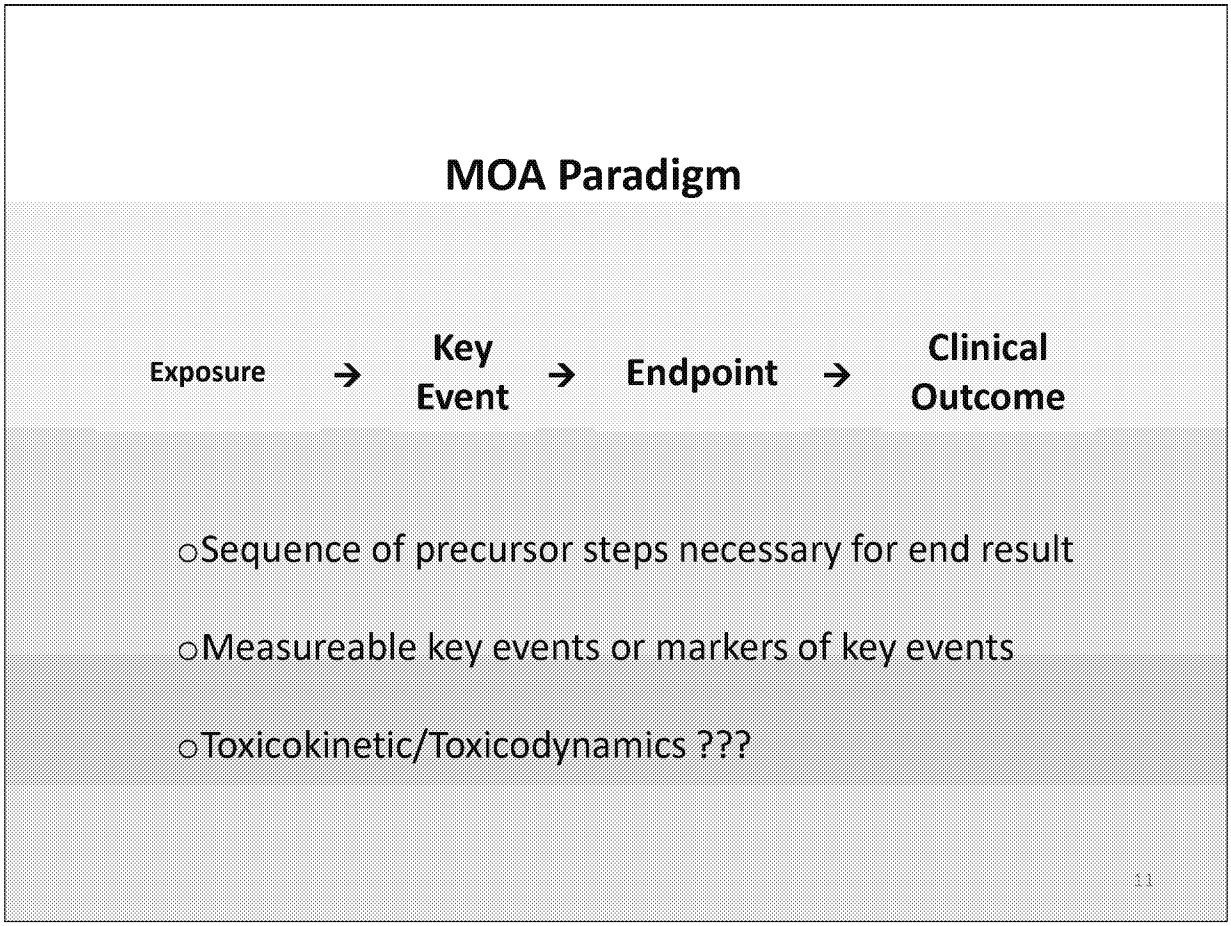
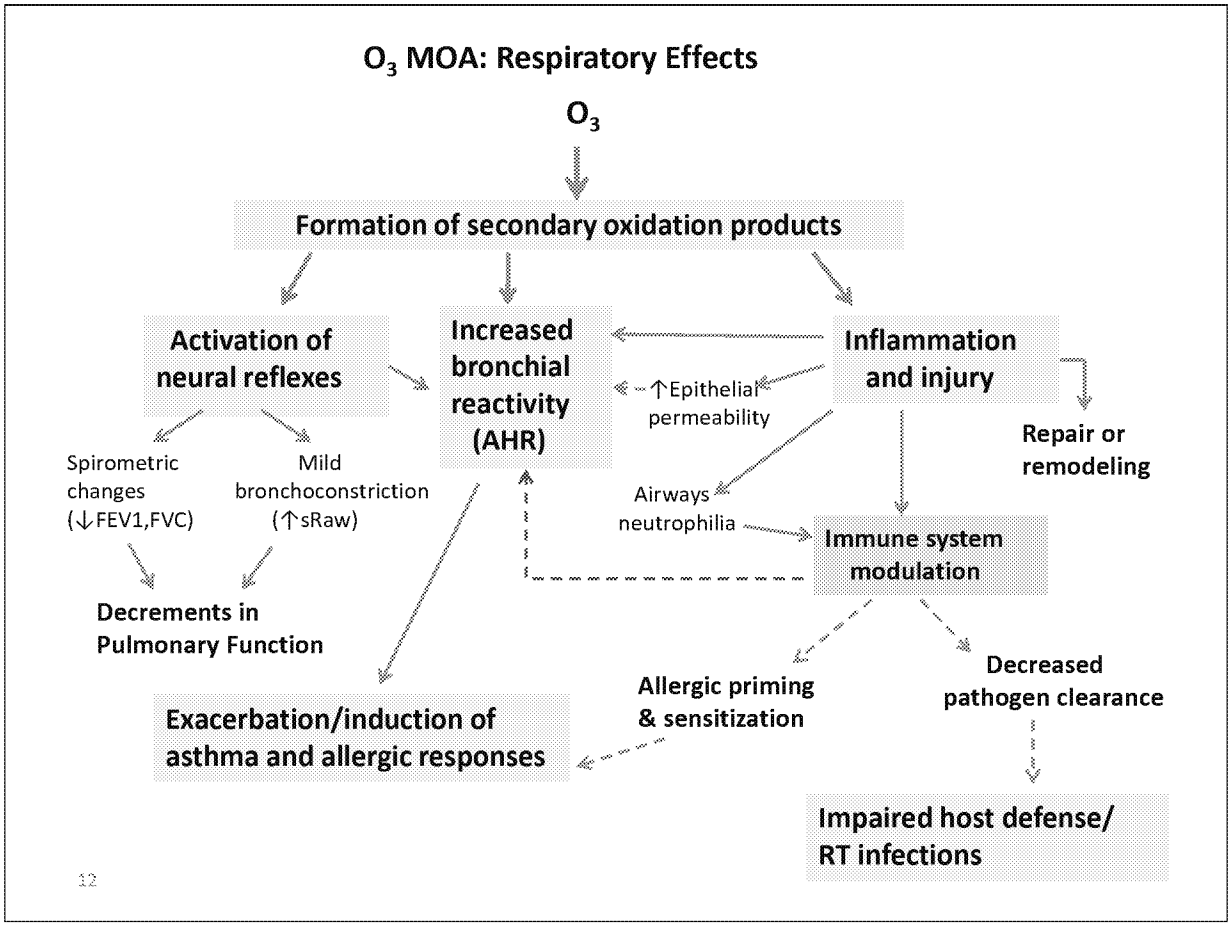
There is a wealth of available data on the criteria air pollutants, including…human and animal, experimental and observational.

For the criteria pollutants we often rely on the evidence from the human studies



These scheme will be familiar to many of you – key elements of the MOA paradigm include ….

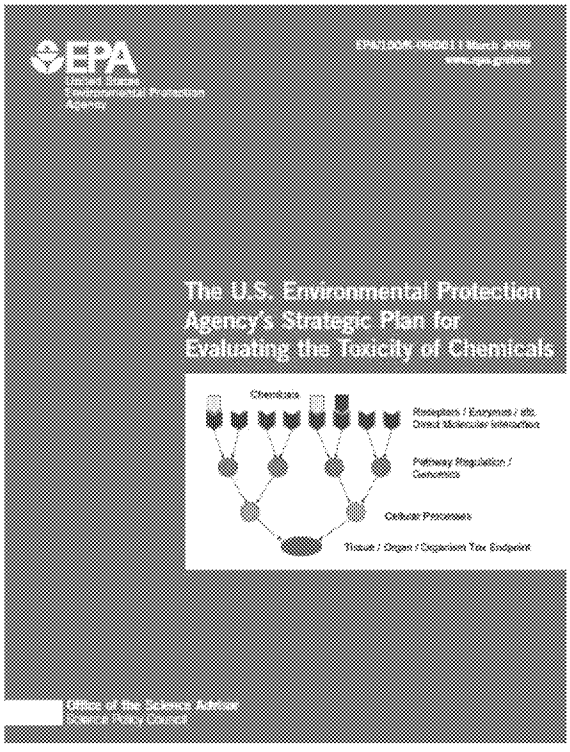


Here is an application of the MOA paradigm for respiratory effects of O3. It begins with inhalation of ozone and its reactions within the extracellular lining fluid in the respiratory tract. This scheme is more complex than simple or linear.

The cause and effect relationships are indicated by the arrows
Dashed and solid lines represent different degrees of certainty about the pathways taking into consideration the strength of evidence from human vs animal studies
As can be seen from this illustration, ozone has multiple MOA and they may be tied to different clinical outcomes.

We could have a long discussion of each of these elements and the weight of evidence for each. This diagram is an illustration, which reflects my views and not a consensus opinion.

Ozone. like other reactive gases, forms secondary oxidation products in the lung lining fluid. This is a key step which is not likely mimicked by in vitro systems



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Moving on to the Toxicity Pathways Paradigm.....there is a great deal of interest by toxicologists in general and by the EPA in particular in developing strategies for evaluating the toxicity of chemicals. The diagram presented on the next slide is taken from a 2009 EPA document based on the NRC report Toxicity in the 21st Century–
Here we have an illustration of the toxicity pathways approach. That is to say...(see box). Keep in mind that perturbations are disruptions of normal biological processes. And that the initial input is the source and the end result is morbidity and mortality.

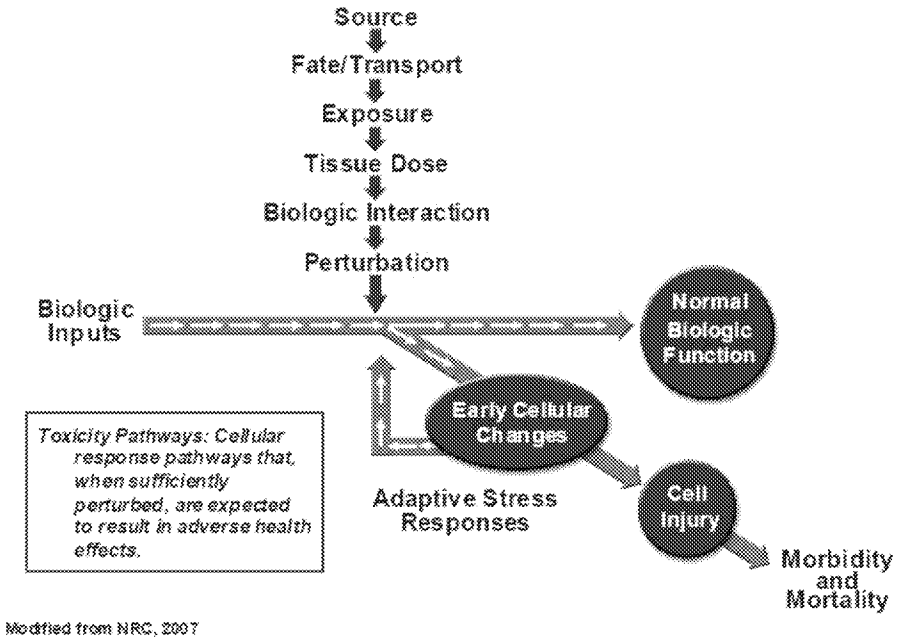

Figure 1. Toxicity Pathways. Toxicity pathways describe the processes by which perturbations of normal biological processes due to exposure to a stressor (e.g., chemical) produce changes sufficient to lead to cell injury and subsequent events (modified from NRC, 2007).

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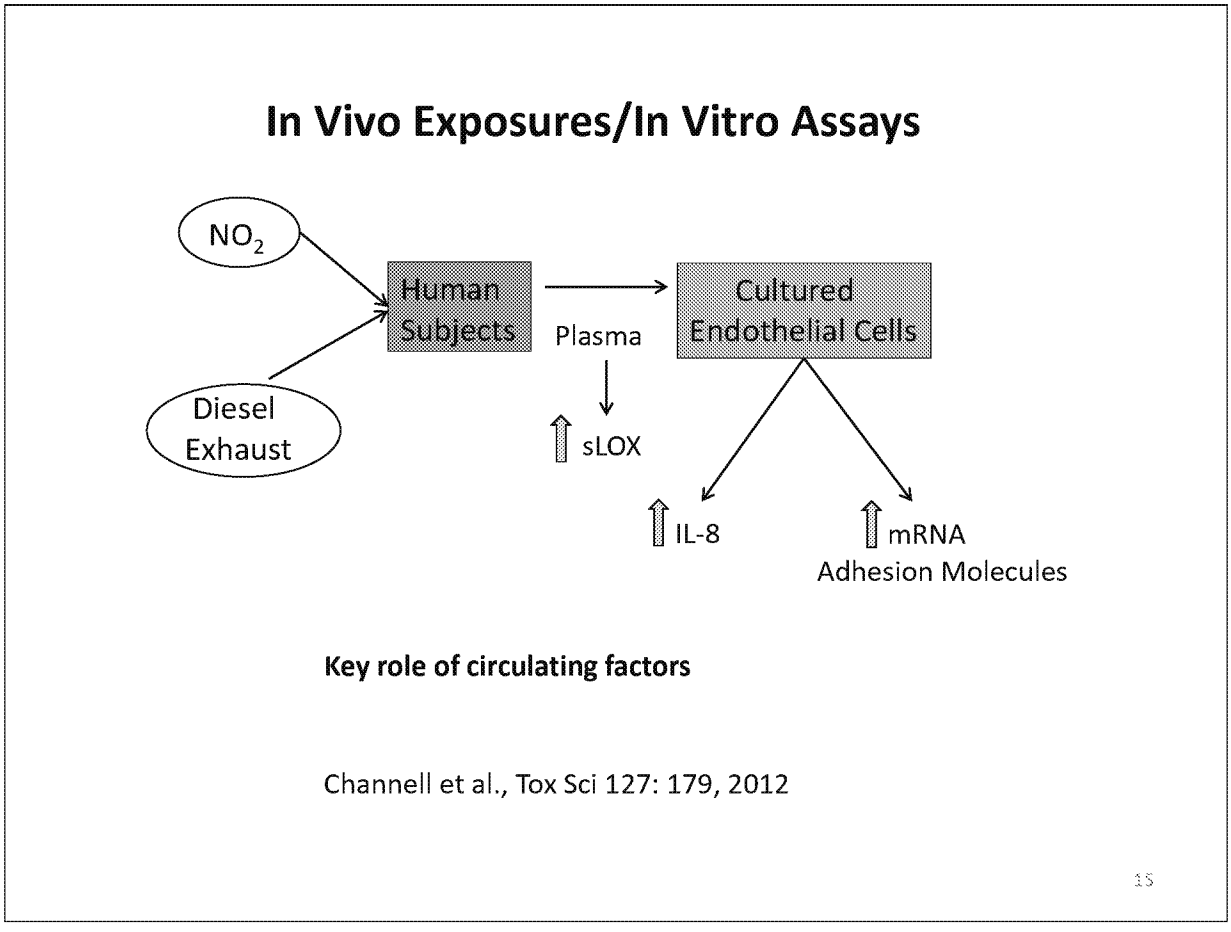
Sequence of precursor steps

Toxicokinetics/toxicodynamics

Perturbation of biological pathways

Adaptive responses

Large gap between cellular events and morbidity and mortality highlights the need for validation/anchoring



A good example of this approach applied to air pollution is found in this recent paper by Channell et al. which combines in vivo exposures of humans with in vitro assays.

Human subjects were exposed to 2 kinds of air pollution (2 h inhalation), blood samples were obtained (immediately after and 24 h after exposure) and plasma samples were prepared. Some of the plasma sample was analzyed for sLOX(soluble lectin like receptor for oxidized LDL). Part was applied to cultured human endothelial cells. Media from the cultured cells was analyzed for pro-inflammatory protein IL-8 and for mRNA of endothelial adhesion markers.

This study demonstrates that circulating factors (that is components in the blood of the volunteers) were altered by exposure to NO2 and diesel exhaust. These circulating factors led to EC activation as measured by this in vitro assay.

This is interesting work , and more is required to link these changes to morbidity.

This approach could be used to evaluate the effects of different mixtures of criteria air pollutants (Diesel exhaust is obviously an example of a mixture, one component of which is NO2). Possible application in our multipollutant work.

Groupings of Pollutants and their Effects

By fundamental biological reactivity

- Oxidative injury
- Affinity for neural receptors
- Recognition by immune cells
- Covalent binding to DNA or proteins

(Mauderly, et al., Inhalation Toxicology 22(S1):1, 2010)

By surrogate marker

- Endothelial function
- Endothelial progenitor cells
- Blood pressure
- ANS measures
- Systemic inflammation
- Insulin resistance

(S. Rajagopalan, AAAR March 2010)

By key events or endpoints in the toxicity pathways

Converging Pathways/Converging Effects

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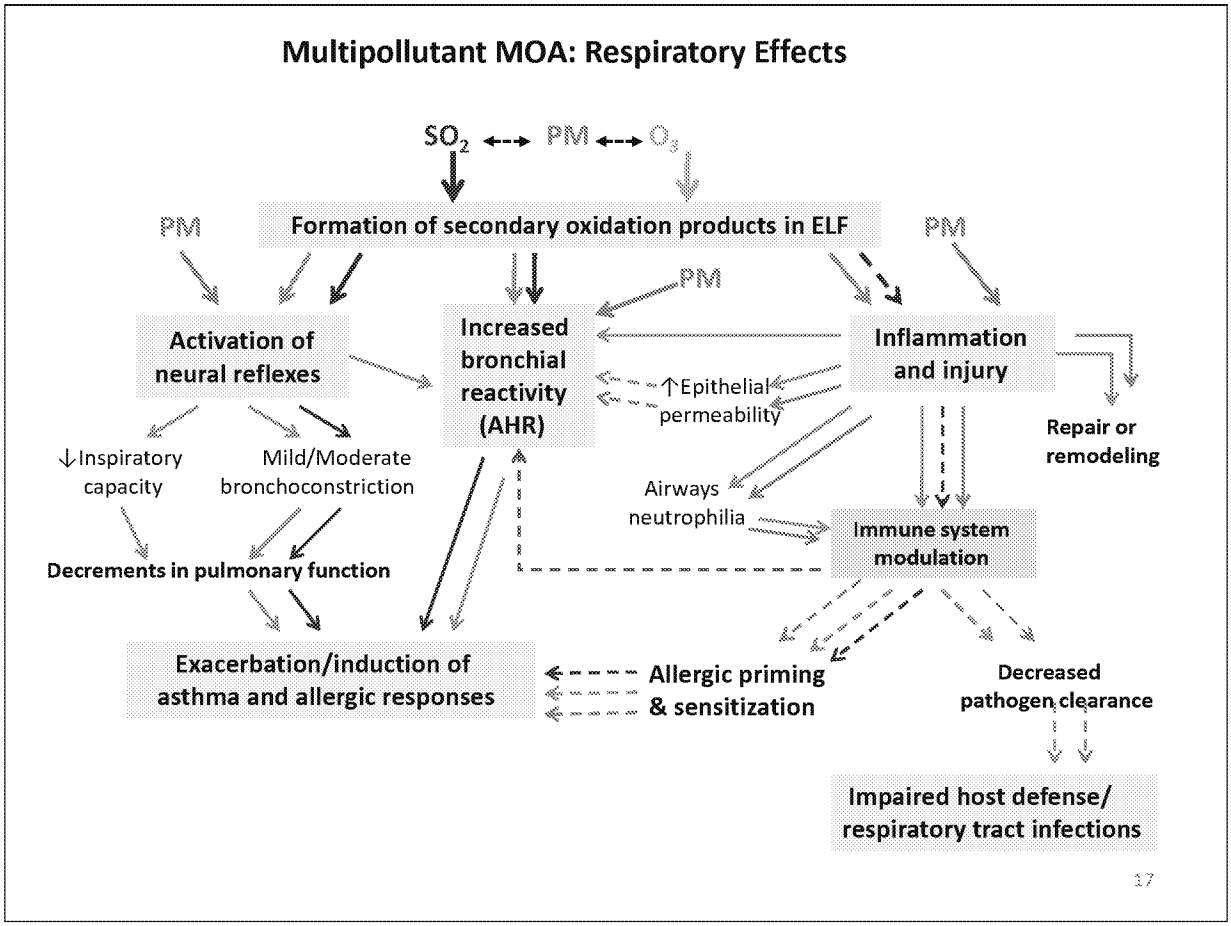
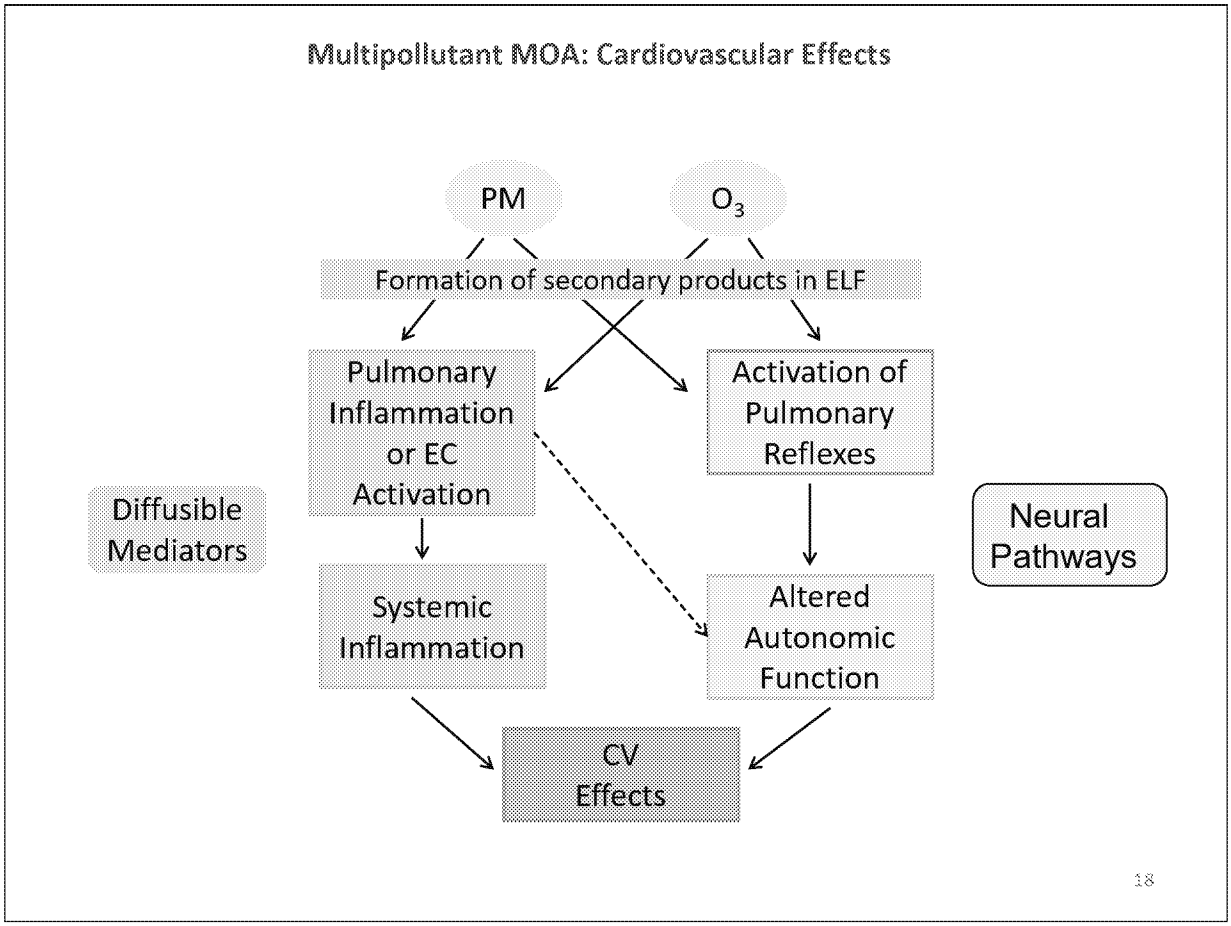
There have been a variety of suggestions regarding how to group pollutants in a multipollutant context. These include grouping by fundamental biological reactivity and by surrogate markers. We can also use a converging pathways or converging effects approach based on MOA, as will be illustrated in the next slides.



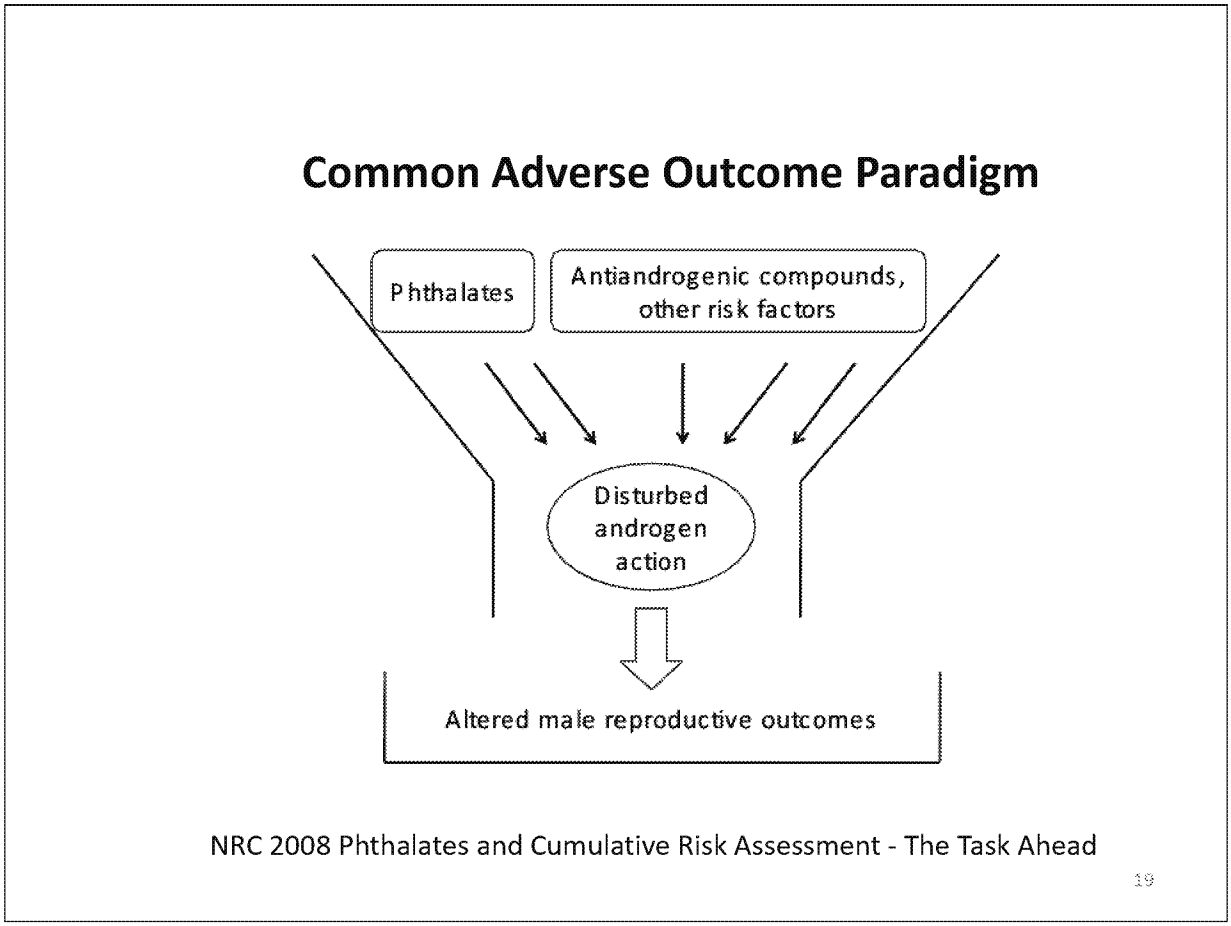
This is an example of a converging pathways approach for SO2, PM and O3.

Note the color coding of the pollutant pathways.

This slide shows a second way to use MOA information in a multipollutant context: converging pathways approach.
Here we are integrating information about 3 criteria air pollutants around toxicity pathways in a key events analysis.
Note that the pathways are color-coded with each color representing a different pollutant.
We can also capture potential additivity and interactive effects in this scheme.
The advantage of this approach is that it enables us to see which pathways have been worked out for each pollutant from start to finish.



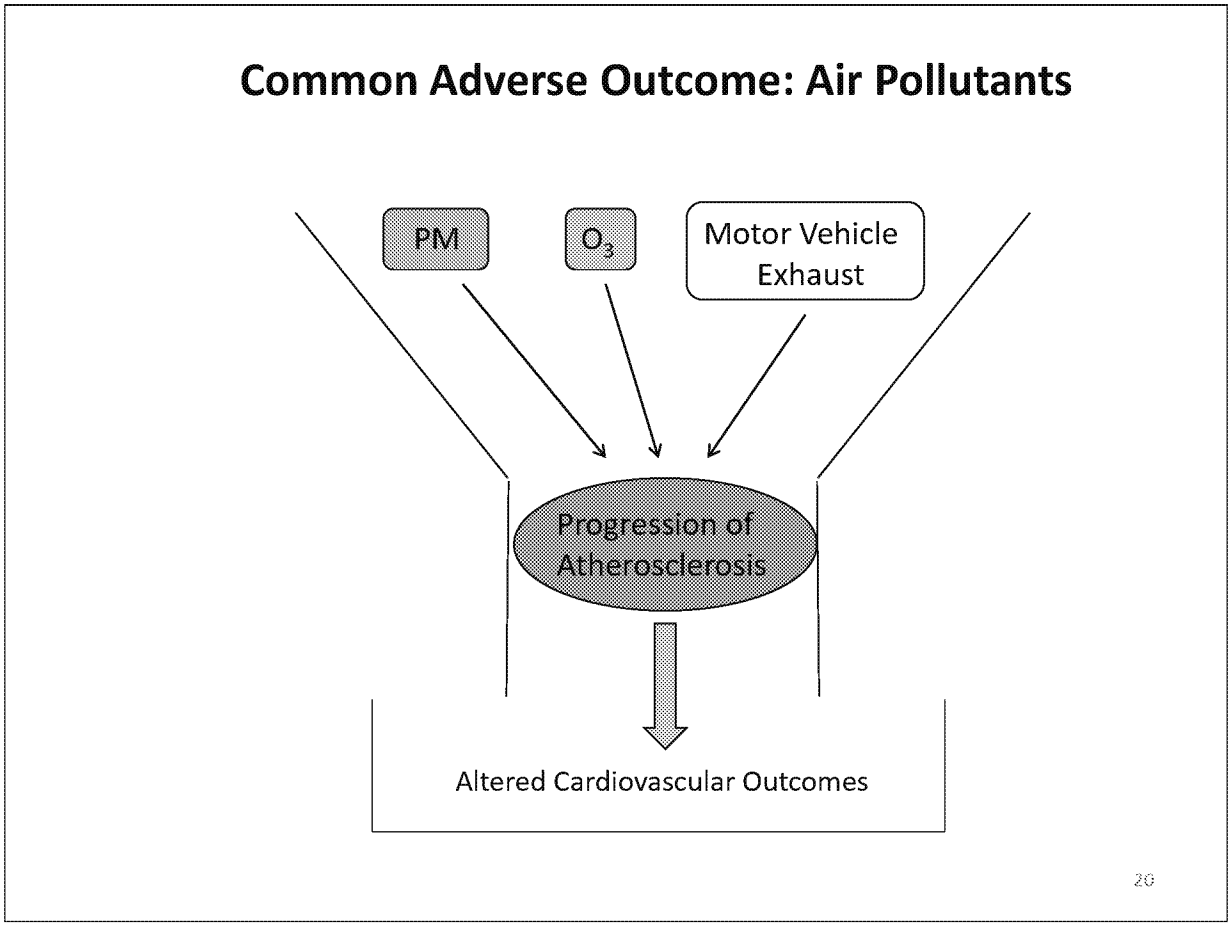
Emerging studies support this converging pathways scheme for PM and O3



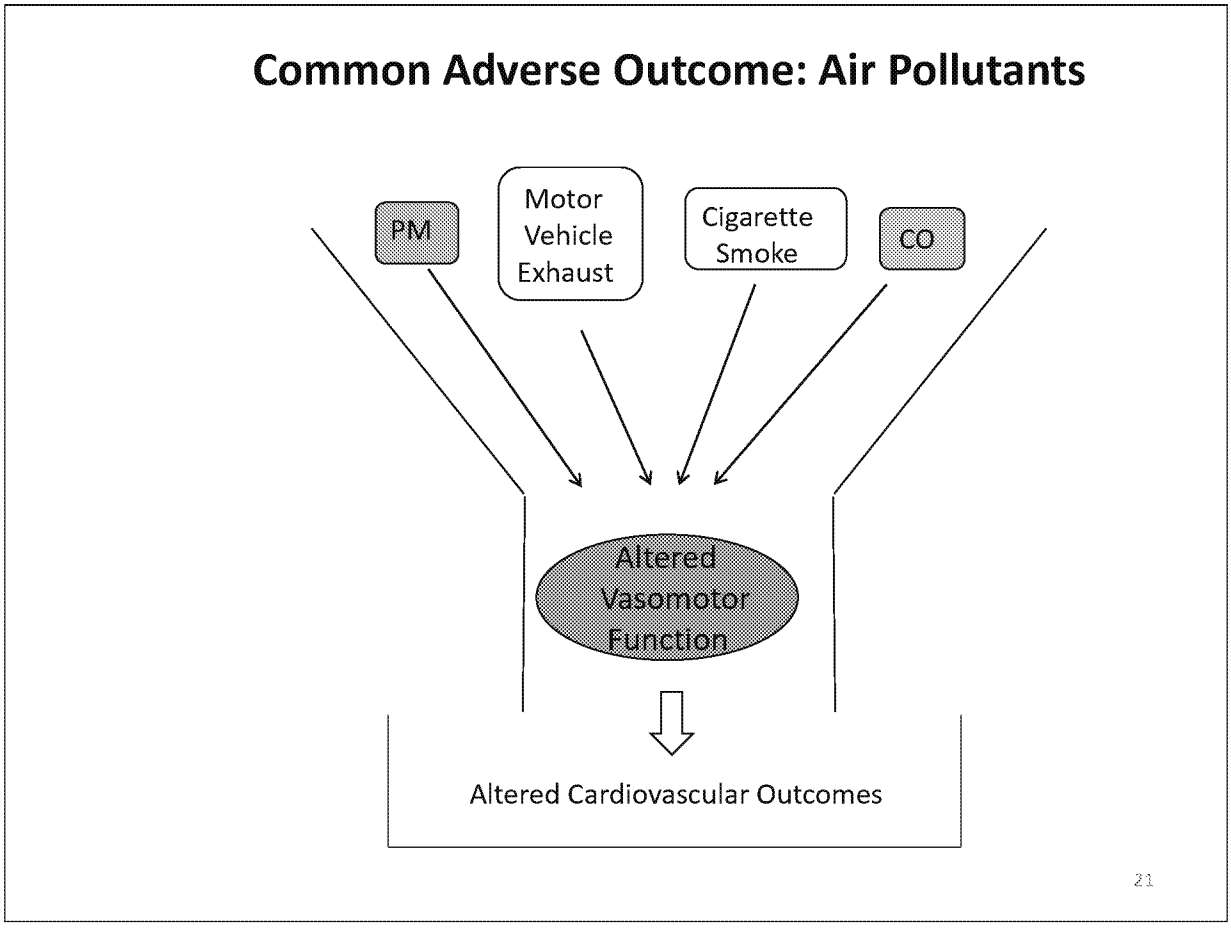
Another paradigm is the Common Adverse Outcome paradigm which was developed by the National Research Council for application to mixtures of phthalates. Here is an illustration showing phthalates and other stressors as the input with altered male repro outcomes as the output

An integration point – disturbed androgen action- links the stressors and the outcome.

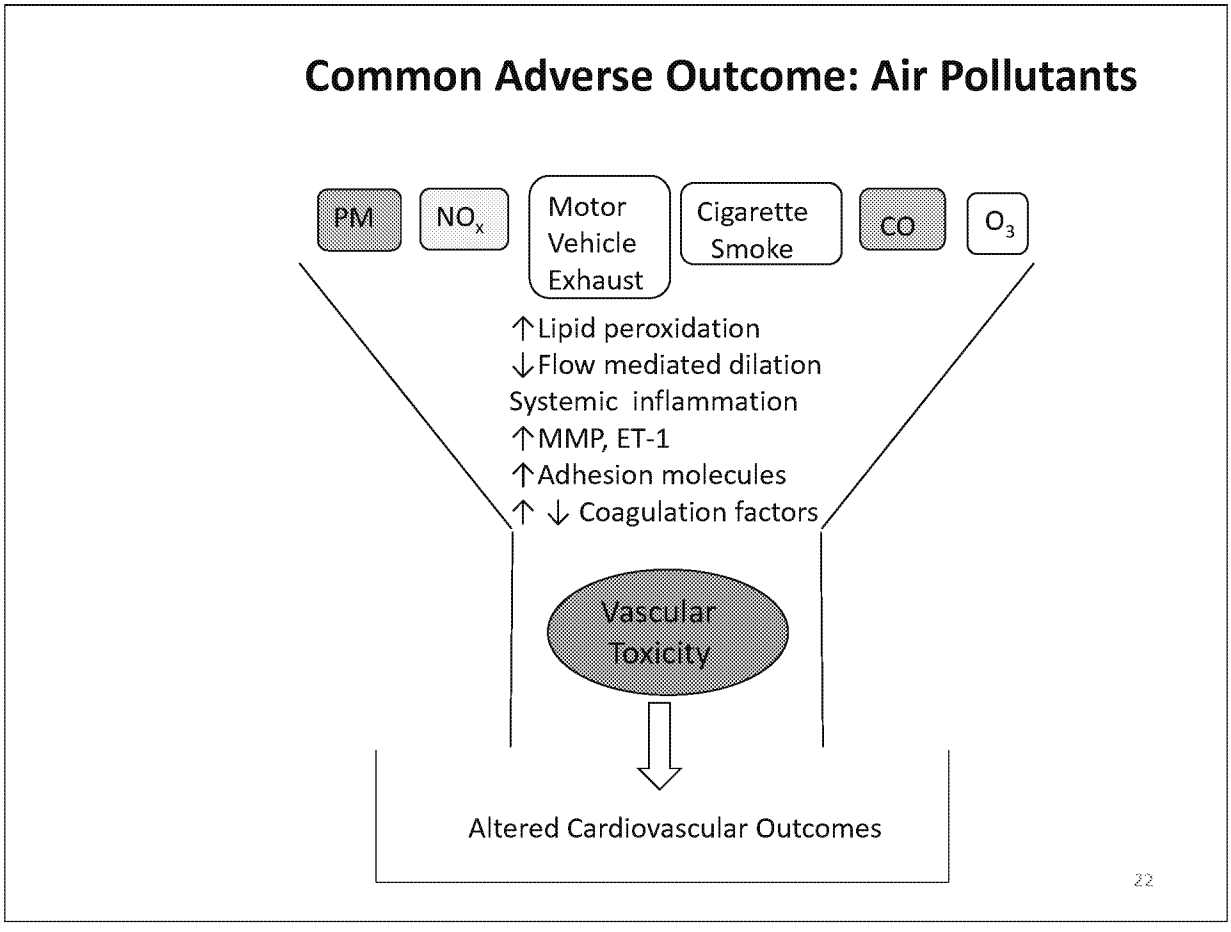
Different mechanisms can contribute to disturbed androgen action – including mechanisms affecting the level of the hormone and the ability of the receptor to respond to hormone



This sort of thinking can be applied to air pollutants and CV effects. In this example the progression of atherosclerosis is the integrating endpoint which links the pollutants to the common adverse outcome.



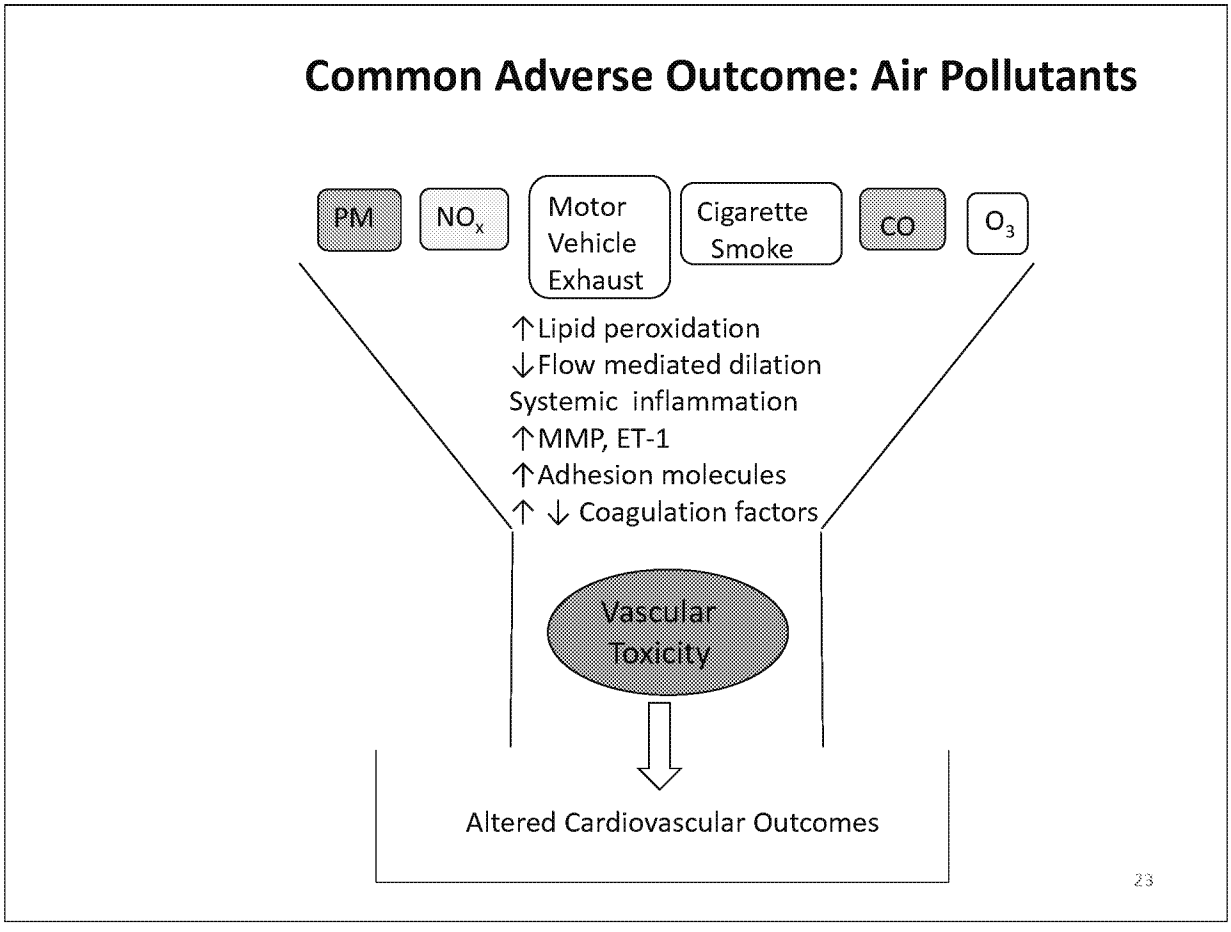
While here Altered Vasomotor Function is the integrating effect.



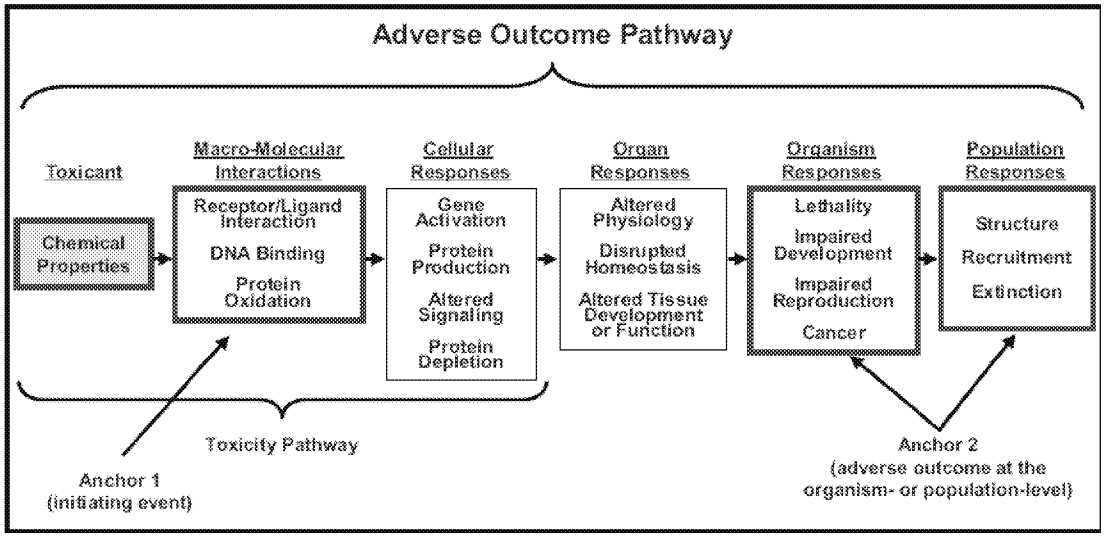
A more detailed scheme shows the various mechanisms which have been demonstrated in air pollution studies that contribute to vascular toxicity.

Vascular toxicity has been linked to altered cardiovascular outcomes.

However Common Adverse paradigm for phthalates was designed for situations where pollutants act on specific molecular targets which is not necessarily the case here.



However, based on available evidence, we may hypothesize that a CAO for air pollutants involves altered NO bioavailability resulting from any of 3 known pathways at the molecular level. This paradigm may prove useful if research points to these specific molecular targets.



Ankley, et al., Env Tox Chem 29: 730, 2010

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The last paradigm which I will discuss in the AOP, developed by a group of EPA scientists for ecotoxicology research and risk assessment A recent paper by Ankley et al., describes this approach.

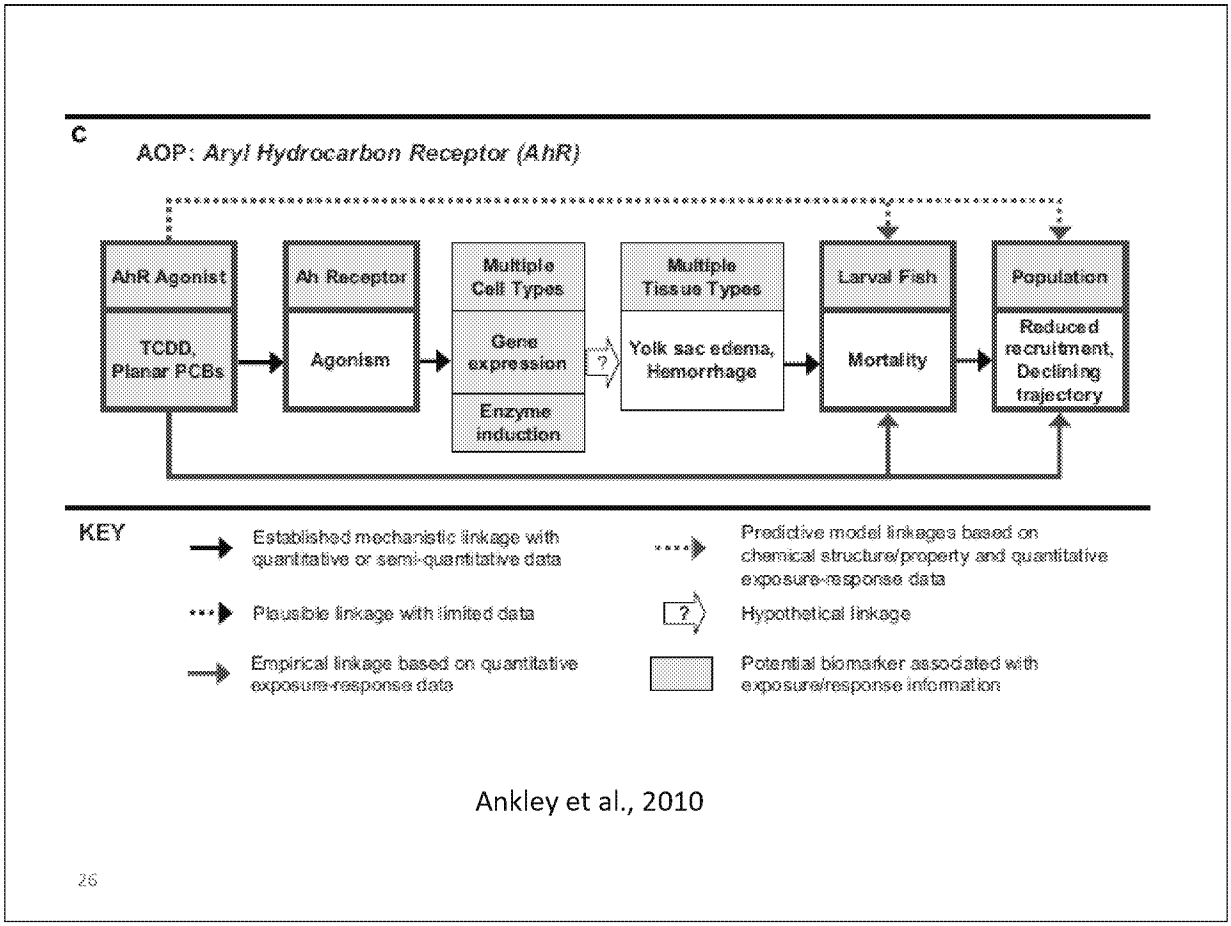
Here is a conceptual diagram of key features of an adverse outcome pathway (AOP). Each AOP begins with a molecular initiating event in which a chemical interacts with a biological target (anchor 1) leading to a sequential series of higher order effects at the cellular, organ, organismal and population levels to produce an adverse outcome with direct relevance to a given risk assessment context...the first three boxes are the parameters that define a toxicity pathway

Clearly illustrates effects over different levels of biological organization. MOA paradigms can also span different levels of biological organization.

"The AOP framework also illustrates how effects caused by mixtures of chemicals that act via the same molecular initiating event...or affect pathways that converge at common intermediate steps …can be aggregated for risk characterization."

"AOPs do not, however, address the question of what dose of chemical will cause sufficient perturbation to drive the pathway to the adverse outcome."

Ankley, et al., 2010

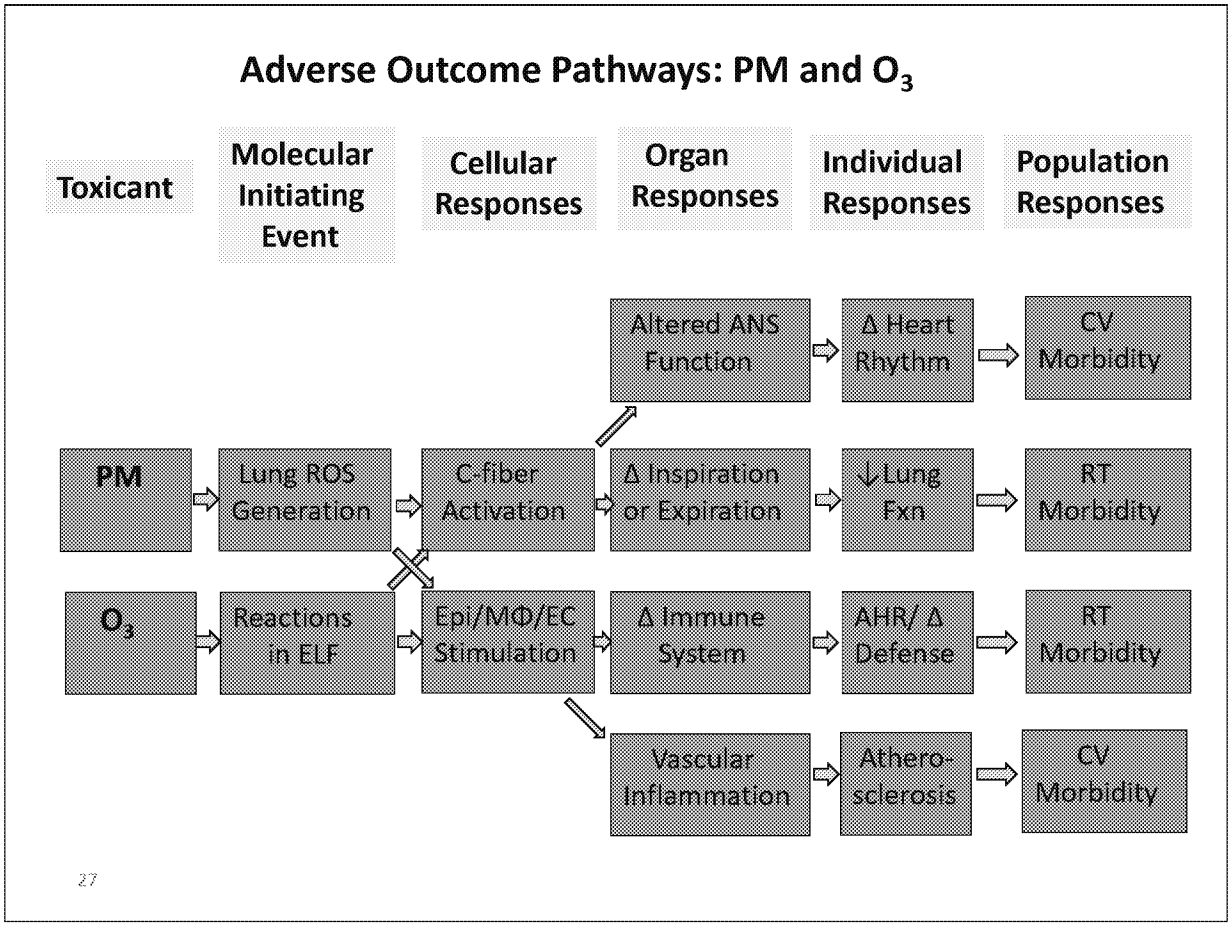


A word about biomarkers and where they may fit into the AOP.

Here the biomarker is at the cellular response level, other levels will also work.

Biomarkers can be defined broadly

The AOP diagram viewed here illustrates biomarkers at the level of organ responses and biomarkers which were not linked to the next level of response



Here is an application of the AOP to criteria air pollutants....
Overlap in cellular responses/common intermediate steps and in the outcome – short and long term exposures here
Connection of molecular initiating event to adverse outcomes

Toxicology 338 (2015) 85–94

Contents lists available at ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

Review

Conceptual model for assessing criteria air pollutants in a multipollutant context: A modified adverse outcome pathway approach

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ARTICLE INFO

Article history:
Received 12 May 2015
Received in revised form 25 June 2015
Accepted 10 July 2015
Available online 18 July 2015

Keywords:
Air pollution
Criteria pollutants
Multipollutant
Adverse outcome pathway

ABSTRACT

Air pollution consists of a complex mixture of particulate and gaseous components. Individual criteria and other hazardous air pollutants have been linked to adverse respiratory and cardiovascular health outcomes. However, assessing risk of air pollutant mixtures is difficult since components are present in different combinations and concentrations in ambient air. Recent mechanistic studies have limited utility because of the inability to link measured changes to adverse outcomes that are relevant to risk assessment. New approaches are needed to address this challenge. The purpose of this manuscript is to describe a conceptual model, based on the adverse outcome pathway approach, which connects initiating events at the cellular and molecular level to population-wide impacts. This may facilitate hazard assessment of air pollution mixtures. In the case reports presented here, airway hyperresponsiveness and endothelial dysfunction are measurable endpoints that serve to integrate the effects of individual criteria air pollutants found in inhaled mixtures. This approach incorporates information from experimental and observational studies into a sequential series of higher order effects.

The proposed model has the potential to facilitate multipollutant risk assessment by providing a framework that can be used to converge the effects of air pollutants in light of common underlying mechanisms. This approach may provide a ready-to-use tool to facilitate evaluation of health effects resulting from exposure to air pollution mixtures.

Published by Elsevier Ireland Ltd.

Background

- Air pollution is a complex mixture of particulate and gaseous components -no two air sheds are exactly alike.
- There is an endless number of unique multipollutant mixtures with little to no information on toxicity.
- Conventional epidemiologic and toxicological approaches are too expensive and low throughput to adequately address this issue.
- Hundreds to thousands of studies have been conducted for the purpose of elucidating underlying changes in genes, biomarkers, proteins, etc.
- The utility of these findings is limited because of the inability to link such changes to an adverse outcome that is relevant to risk assessment.
- Mechanistic data may be most informative for risk assessment when translated into measurable changes including organ responses, clinical consequences, and impacts to the population at large.

Goals

- Develop a conceptual model for air pollution mixtures that links initiating events at the cellular and molecular level to population-wide impacts.
- Identify measurable endpoints which serve to integrate the effects of individual criteria air pollutants found in inhaled mixtures
  - **Airway hyperresponsiveness is a key feature of asthma**
  - **Endothelial dysfunction is a risk factor for cardiovascular (CV) disease**
  - **Both are physiological changes at the organ level which can be measured in the clinic/laboratory**
- Incorporate information from experimental and observational studies into a sequence of steps occurring over multiple levels of biological organization

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Lots of epi studies for criteria air pollutants – population level responses
Intermediate endpoints are measurable in the clinic and linked to the population level responses
Much of the information came from the ISAs

Case Report 1:

Irritant gases, airway responsiveness and respiratory morbidity

Airway responsiveness reflects the sensitivity of airway smooth muscle to natural or pharmacological stimuli

Epidemiologic Studies

- **Short –term exposures and associations with:**
  - Respiratory symptoms
  - Asthma medication use
  - Respiratory-related ED visits
  - HA including those for asthma
- **Long-term exposures and associations with:**
  - Respiratory symptoms
  - Bronchitis
  - Asthma
  - New onset asthma
- **Potential co-pollutant confounding for both short- and long-term studies but more evidence for independent effects in short-term studies**

Case Report 1

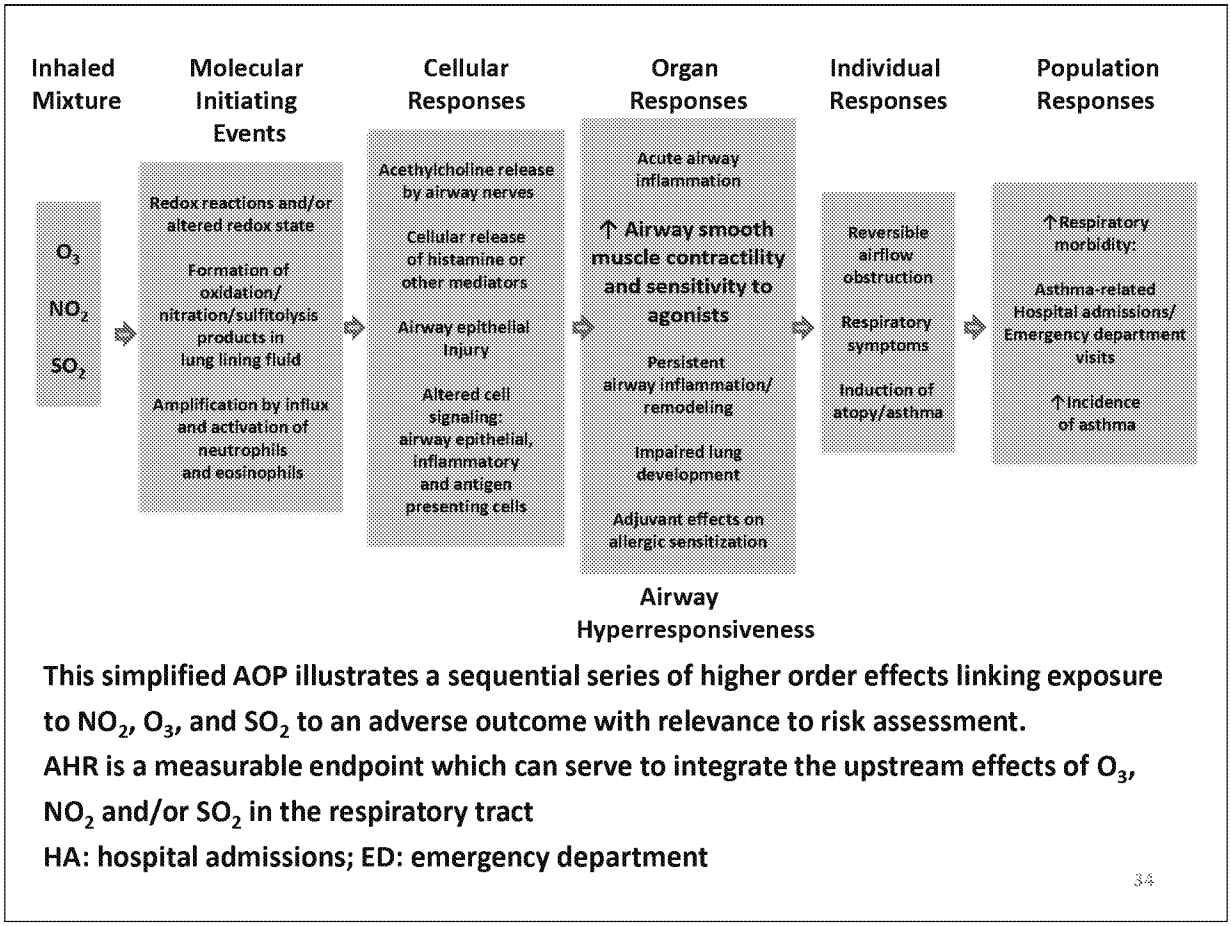
Controlled Human Exposure Studies

- Secondary oxidation products in the lung lining fluid
- ↑ immune responses in healthy individuals
  - Neutrophil influx in airways
  - Th2 polarization (repeated exposures)
  - Altered cell surface molecules on monocytes that are characteristic of innate immunity and antigen presentation
- ↑ immune responses in allergic asthmatics
  - Eosinophils, ECP in lung lining fluid
  - Pro-allergic cytokines
  - Activation of the TLR4 pathway
- Physiologic changes in airway smooth muscle (healthy and asthmatics)
  - ↑ airway resistance due to ACh release by airway nerves and to inflammatory mediators
- ↑Inherent reactivity of airway smooth muscle (healthy and asthmatics)
  - AHR following a direct or allergen challenge

Case Report 1

Toxicological Studies

- Secondary oxidation products in the lung lining fluid
- ↑ immune responses
  - Allergic sensitization in naïve animals
    - Activation of the TLR4 pathway
    - Dendritic cell maturation
    - Polarization to Th2 and Th17 phenotype
  - Enhanced allergic responses in allergen-sensitized animals
- Physiologic changes in airway smooth muscle
  - ↑airway resistance due to neural reflexes involving vagus nerve
- ↑Inherent reactivity of airway smooth muscle (e.g. AHR)
  - Hyperreactivity of vagal nerves due to inflammatory mediators
  - Stimulation of local axon reflexes with release of tachykinins
  - Mast cell degranulation
  - Airway remodeling
  - Disruption of the epithelial-mesenchymal unit during lung development



Both short and long term exposures integrated in this slide

Case Report 2

PM and O_3, endothelial dysfunction and CV disease

Endothelial dysfunction is defined as impaired blood vessel response to specific vasodilators. It can occur in conduit arteries and microvascular resistance vessels.

Epidemiologic Studies

- Associations between exposure to PM and CV morbidity/mortality
- PM may play a role in triggering acute events or initiating responses that cause disease or promote its progression
  - Short-term PM exposures related to acute MI
  - Long-term PM exposures related to atherosclerosis
  - Some evidence that PM exposure is associated with endothelial dysfunction
- Associations between exposure to O_3 and CV disease
- Less evidence for a relationship between O_3 exposure and endothelial dysfunction

Case Report 2

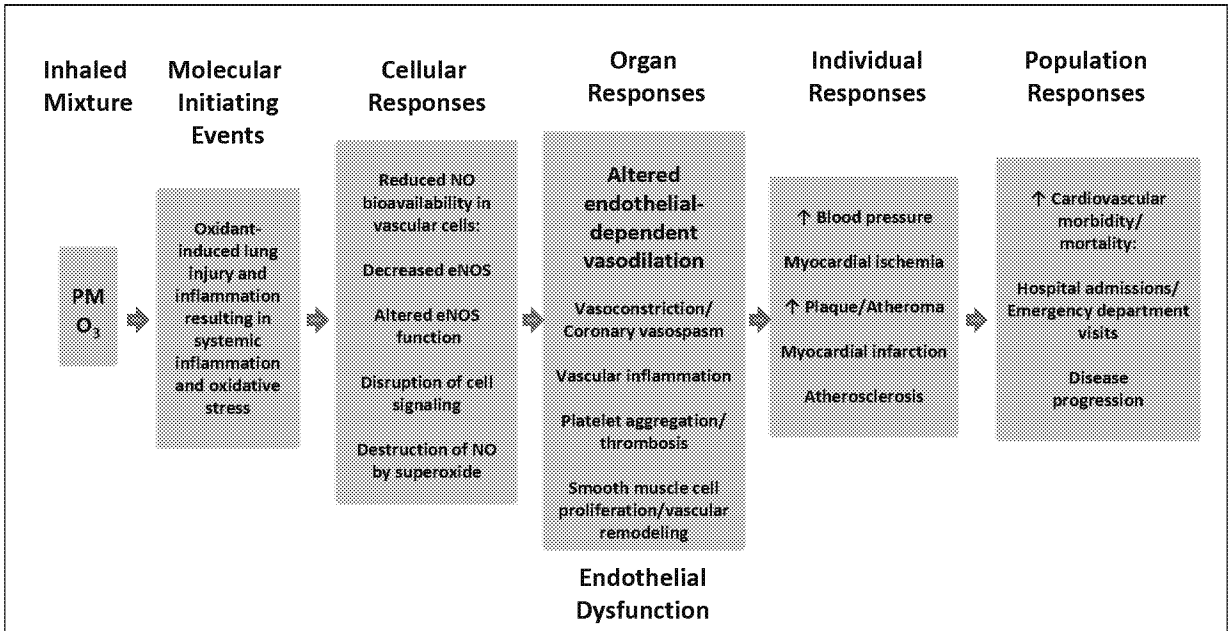
Controlled Human Exposure Studies

- Evidence for PM-mediated endothelial dysfunction in some studies
- Lack of evidence for O_3-mediated endothelial dysfunction in healthy subjects

Toxicological Studies

- PM and O_3 both mediate endothelial dysfunction

Principal pathway underlying endothelial dysfunction is loss of endothelial nitric oxide which can happen by several mechanisms



This simplified AOP illustrates a sequential series of higher order effects linking exposure to PM and O_3 to an adverse outcome with relevance to risk assessment.
Cellular responses refer to responses in all vascular cell types.
Endothelial-dependent vasodilation is a measurable indicator of endothelial.
dysfunction which can serve to integrate the upstream effects of PM and O_3 in the vasculature.
NO: nitric oxide; eNOS: endothelial nitric oxide synthase; BH_4: tetrahydrobiopterin

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Both short and long term exposures integrated in this slide

Relevancy

The proposed model has the potential to facilitate multipollutant risk assessment by:

- **Providing a framework that can be used to converge the effects of air pollutants based on common underlying mechanisms**
- **Identifying data gaps**
- **Enabling prioritization of targeted research in the most efficient and cost-effective manner possible**
- **Allowing the incorporation of biomarker data that is predictive of clinically significant outcomes**

Limitations

- Population effects may be mediated by alternative mechanisms than the ones identified
- Model does not account for adaptation and repair
- Model does not address dose-response considerations
- Toxicokinetic and toxicodynamics data have not been incorporated

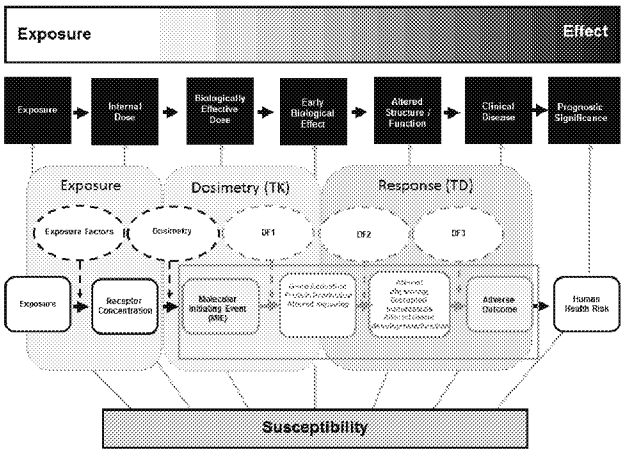
"Dosimetry links exposure and response"

Annie Jarabek

What a Difference the Dose Makes
Webinar Series
April 28, 2016

Advancing AOP and MOA

- **Need to define different dose metrics in order to apply key events of adverse outcome pathways (AOP) and mode of action (MOA) in risk assessment**
  - Screening dosimetry insufficient for quantitative response analysis
  - Portal-of-entry descriptions
  - Broad context re: both endpoints and chemical classes
- **Support transparency, causal linkage and interoperability along continuum: exposure to dose-response analysis**



Source: US EPA Human Health Risk Assessment (HHRA) FY16-19 Strategic Research Action Plan
https://www.epa.gov/research/strategic-research-action-plans-2016-2019

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Multi-scale modeling to QUANTITATIVELY describe dose metrics associated with key events is required to advance AOP and MOA concepts in risk assessment. Figure shows NRC biomarker scheme of 1989 with AOP and MOA. Biomarkers at different levels of biological organization serve to link MIE and KE, and describe pathogenesis. NOTE: Dosimetry (TK) links exposure and response! Interoperability along E-D-R continuum advocated by SOT CCT (2012) workshop to break down silos among diverse disciplines and support seamless integration of models to best be able to evaluate risk management decisions

Impact

- This approach may provide a ready-to-use tool to facilitate the evaluation of health effects resulting from exposure to air pollution mixtures.
- Evidence from epidemiologic, controlled human exposure and toxicological studies of single criteria pollutants can be utilized to develop AOPs for mixtures of these pollutants.
- AOPs may be simplified, as illustrated here, or more detailed, including multiple effects occurring in multiple compartments at each level of biological organization.
- In addition, they may be used to indicate the certainty of mechanistic linkages between steps and to portray potential biomarkers of exposure or effect.
- Further, AOPs may allow the incorporation of toxicodynamic, toxicokinetic and biomarker data into a conceptual model.
- This may facilitate the quantitation of exposure-response relationships for multipollutant mixtures.

Future Direction

The outputs from this project may impact the reviews of the National Ambient Air Quality Standards.

This work may also move us one step closer to explicit consideration of multipollutant evidence in the standard setting process.

Questions?